HHS Public Access

Author manuscript

J Anal Toxicol. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:

J Anal Toxicol. 2016 May ; 40(4): 248–254. doi:10.1093/jat/bkw015.

Quantification of Hydrazine in Human Urine by HPLC-MS/MS

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Abstract

Currently used on F-16 fighter jets and some space shuttles, hydrazine could be released at toxic levels to humans as a result of an accidental leakage or spill. Lower-level exposures occur in industrial workers or as a result of the use of some pharmaceuticals. A method was developed for the quantitation of hydrazine in human urine and can be extended by dilution with water to cover at least six orders of magnitude, allowing measurement at all clinically significant levels of potential exposure. Urine samples were processed by isotope dilution, filtered, derivatized, and then quantified by HPLC-MS/MS. The analytical response ratio was linearly proportional to the urine concentration of hydrazine from 0.0493 to 12.3 ng/mL, with an average correlation coefficient R of 0.9985. Inter-run accuracy for 21 runs, expressed as percent relative error (% RE) was ≤ 14%, and the corresponding precision, expressed as percent relative standard deviation (% RSD) was ≤ 15%. Since this method can provide a quantitative measurement of clinical samples over six orders of magnitude, it can be used to monitor trace amounts of hydrazine exposure as well as industrial and environmental exposure levels.

Keywords

hydrazine; *p*-anisaldehyde; quantitative HPLC-MSMS; isotope dilution; aviation

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Supplementary Data

Supplementary data is available at *Journal of Analytical Toxicology* online.

Disclaimer

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Conflict of Interest Disclosure

The authors declare no competing financial interest.

Introduction

Hydrazine (HZ, H_2N-NH_2) is a toxic compound and a suspected carcinogen [1,2] that is used as a propellant in rocket fuel and in industry or synthetic applications. HZ was used as fuel during World War II on rocket-powered fighter planes [3]. More recently, the Curiosity rover which landed on Mars in 2012 used a HZ-fueled rocket engine for terminal descent [4]. HZ was used as fuel in auxiliary power units on some space shuttles [5] and is used in emergency power units, including in the F-16 fighter jet [6,7]. The titan rocket family used a mixture of HZ and dimethyl HZ from 1959 to 2005. This family of rockets was retired due to the high cost and special care required in the use of HZ fuel [8]. HZ is also used as a synthetic intermediate in the polymer, pharmaceutical, and agricultural industries. [9–15]. Some active pharmaceutical ingredients are known to form HZ upon metabolism, including hydralazine which is used to treat severe high blood pressure and isoniazid, which is used to treat tuberculosis. [16–19] Isoniazid-induced hepatotoxicity is a known side-effect of the drug, and it has been speculated that this side effect is due to the hepatotoxic metabolite HZ [19–22]. HZ is used in the treatment of wastewater for dehalogenation and is also a known byproduct of chloramination [23–27]. Trace amounts of hydrazine have been reported previously in chloraminated drinking water samples [23,24].

A case of human ingestion of HZ was documented in 1965 by James Reid in Africa, who treated a sailor who had accidentally ingested a HZ solution of unknown concentration. The HZ solution was on board for prevention of corrosion to the ship's boiler. The sailor mistook the HZ solution for a glass of water. After ingestion, his symptoms included vomiting, unconsciousness, dilated pupils, and shallow irregular breathing. The patient was admitted to a hospital, and his condition improved when treated with assisted respiration under anesthesia. The patient improved enough to be discharged two weeks following the incident, but follow-up monitoring of the patient was not available [12]. In 2000, a cancer patient self-medicated with hydrazine sulfate for 4 months after diagnosis. The patient was admitted to a hospital for hepatorenal failure and passed away after a week of treatment [28].

Human exposure to HZ can occur accidently as described above or in the leakage or spill of rocket fuel propellant. Repeated exposure to HZ may also occur among industrial workers that routinely handle HZ products [11, 29]. The permissible exposure limit (PEL) of HZ, set by the Occupational Safety and Health Administration (OSHA), is currently 1 ppm [2]. The American Conference of Governmental Industrial Hygienists (ACGIH) guideline for threshold limit value – time weighted average (TLV-TWA) is currently 0.01 ppm for hydrazine [2]. The National Institute for Occupational Safety and Health ceiling recommended exposure limit (NIOSH REL) is currently 0.03 ppm [2]. A study of Japanese workers exposed to HZ in an industrial setting, where the exposure level was approximately 0.1 ppm HZ in air, revealed that the workers excreted an average of 2.7 ± 2.0 ppm ($n=8$) HZ in their urine [29]. Based on this study, occupational exposures are expected to produce urine excretions greater than 1 ppm HZ. Low level exposure as a result of the use of certain pharmaceuticals may be of interest for biomonitoring but is not expected to mimic the concentrations expected from occupational or industrial exposure. The concentration of HZ that is immediately dangerous to life or health (IDLH) is based on animal studies and is currently 50 ppm [2, 30]. In a study where rats ingested toxic amounts of HZ, an average of

70.2 (± 14.9) ppm HZ was excreted in their urine [31]. Although there is no data for human exposure to HZ exceeding IDLH levels of hydrazine, an acute exposure event would be expected to exceed the upper end of the excreted concentrations observed in the industrial exposure previously reported [29]. Therefore concentrations above 10 ppm are expected to be observed in urine in the case of an event-level exposure.

This work provides a specific HPLC-MS/MS method for the quantitation of HZ in human urine that ranges from the biomonitoring of trace amounts up to event-level exposures. HZ is largely metabolized to nitrogen (N_2), ammonia (NH_3), urea (CH_4N_2O), and other metabolites, but about 10% of the dose is excreted unchanged in urine [31,32]. Since HZ is partially excreted unchanged, exposure to the toxin can be evaluated in a sample of urine from an individual. A method that is both selective and sensitive for HZ in clinical samples is needed and can be achieved with isotope dilution high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). Urine samples were filtered and derivatized followed by quantification using HPLC-MS/MS. With a two-minute run time, this method provides a time-to-first result of less than 2.5 hours, including a calibration curve and quality control samples.

Materials and Methods

Chemicals

Commercially available hydrazine (HZ) sulfate salt (P/N 16046), $^{15}N_2$-HZ sulfate (P/N 489735), *p*-anisaldehyde (P/N 97063), and formic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade solvents acetonitrile, methanol and water were obtained from Fisher Scientific (Pittsburgh, PA, USA). Deionized (18 MΩ) water was used when specified.

Safety Considerations

HZ is a known mutagen and a probable human carcinogen [1–2,9]. HZ should always be handled in a chemical fume hood.

Preparation of stock solutions

HZ sulfate and $^{15}N_2$-HZ sulfate were weighed at milligram quantities using a Mettler Toledo XP6 microbalance (Columbus, OH, USA). Hydrazine concentrations were calculated based on dissociation of hydrazine sulfate in water as previously reported. [15] $^{15}N_2$-HZ was prepared at 123 ng/mL in water, dispensed in one-time use aliquots and stored at −70 °C. HZ sulfate was dissolved in water, dispensed in one-time-use aliquots and stored at working stock solutions of 1.23–307 ng/mL HZ (or 38.4 nM-9.58 μM HZ) at −70 °C. QC high, mid and low range samples were prepared at concentrations of 2.96, 0.887, and 0.296 ng/mL HZ (or 92.3, 27.7, and 9.24 nM HZ) in human urine and stored in one-time-use aliquots at −70 °C. Pooled human urine was purchased from SPEX CertiPrep (Metuchen, NJ, USA). The urine products used were acquired from commercial sources, and the work did not meet the definition of human subjects as specified in 45-CFR 46.102 (f).

Sample processing

For all calibrators, 100 μL of water/acetonitrile (4/1, v/v) was added to a MultiScreen 96-well Ultracel-10 filter plate (10 kDa, MAUF1010, EMD Millipore Billerica, MA) followed by 4 μL of the appropriate working stock of HZ, 4 μL of internal calibrator stock solution of $^{15}N_2$-HZ, and 100 μL aliquot of sample matrix (pooled human urine). Using a shallow 96-well plate as a receiving plate, the filter plate was vortexed at 500 rpm for 10 seconds and then centrifuged at 3,000 × g for 30 minutes at 10 °C. Clinical samples and QC samples were processed similarly but without the addition of HZ calibrators.

Sample preparation

To the filtrate, 200 μL of 20.5 mM *p*-anisaldehyde in methanol was added to each sample and allowed to react under a stream of N_2 gas in a Porvair UltraVap Blowdown Evaporator (Porvair Sciences, Wrexham Wales, UK) at 60 °C for 30 minutes. Once dry, samples were dissolved in 100 μL mobile phase acetonitrile/water (60/40, v/v), 0.1% formic acid and transferred to a 96-well PCR plate (Eppendorf, Hamburg, Germany or equivalent).

HPLC-MS/MS

HZ concentrations in human urine were determined on an AB Sciex 4000 triple quadrupole instrument (AB Sciex, Framingham, MA, USA). The instrument was tuned and calibrated monthly over a mass range of *m/z* 118–2720 using an Agilent ESI tuning mixture (P/N G2421A). Conventional HPLC elution was performed using an Agilent 1200 HPLC system (Agilent, Santa Clara, CA, USA). Samples (5.0 μL) were injected onto an Agilent Zorbax SB-C18 Rapid Resolution HT column (2.1 × 50 mm, 1.8 μm) equipped with an Agilent low-dispersion in-line filter (2 μm frit). Column and autosampler temperatures were 60 °C and 10 °C, respectively. An isocratic separation of acetonitrile/water (60/40), 0.1% formic acid was used at a constant 500 μL/min flow rate and average back pressure of 200 bar. The following optimized instrument parameters were applied for the detection of the analyte and internal standard: collision gas 7 psig; curtain gas 10 psig; ion source gas 1 60 psig; ion source gas 2 60 psig; ion spray voltage 4800 V; temperature 500 °C; collision exit potential 8 V; declustering potential 50 V; entrance potential 10 V; dwell time 32.5 ms; and a ‘unit’ resolution of 0.7 amu at full width half max. Quantitation was determined by MRM (HZ quant. ion *m/z* 269.1 → 134.1, collision energy 28.6 V; HZ confirmation ion *m/z* 269.1 → 136.1, collision energy 26.9 V; $^{15}N_2$-HZ *m/z* 271.1 → 135.1, collision energy 28.6 V) in ESI positive ion mode.

Data acquisition and processing

Data acquisition and quantitative spectral analysis were carried out utilizing AB Sciex Analyst v.1.4.2 build 1346. Percent relative error was reported as $\%RE=[(C_e-C_t)/C_t]\times100$ where C_e is the experimental concentration determined from the calibration curve slope, and C_t is the theoretical concentration. Percent relative standard deviation $\%RSD=(SD/C_{avg})\times100$ was calculated as a measure of assay precision, where C_{avg} is the average concentration calculated, and SD is the standard deviation. Peak area ratios of HZ/$^{15}N_2$-HZ were plotted versus concentration to construct calibration curves of a series of six HZ urine standards. Each standard was injected (*n=21*) and validated over the concentration range of

0.0493–12.3 ng/mL (or 1.5–384 nM). Human urine QCs were prepared at 0.296, 0.887 and 2.96 ng/mL (or 9.2, 27.7, and 92.3 nM) and injected alongside calibrators. QC characterization was completed over the course of six weeks, with three analysts participating and no more than two curves run per day [33]. The ratio of the response for a urine sample to the response for a water sample was used to determine the method recovery in urine as compared to water.

Convenience sample set

Commercially available urine was purchased from Tennessee Blood Services (Memphis, TN, USA) in order to evaluate baseline responses. The acquired urine was from 100 random, anonymous individuals representative of a normal population. As such, the work did not meet the definition of human subjects research as specified in 45-CFR 46.102 (f).

Results and Discussion

Detection and Separation

HZ is a low molecular-weight compound, and derivatization was needed for quantitation with HPLC-MS/MS. Derivatization was carried out with p-anisaldehyde which reacts 2:1 with HZ (Figure 1a). The predominant product ions from fragmentation of derivatized HZ (*m/z* 269.1) were *m/z* 134.1 and 136.1 (Figure 1b). Quantitation of HZ was based on the transition *m/z* 269.1 → 134.1, and confirmation by *m/z* 269.1 → 136.1. The isotopically-labeled standard HZ* ($^{15}N_2$-HZ) also reacts with p-anisaldehyde and was analyzed by the transition *m/z* 271.1 → 135.1. An isocratic separation was used for the reversed-phase HPLC separation, with a mobile phase composition of 40/60 (v/v) water/acetonitrile with 0.1% formic acid. Under these conditions, derivatized HZ was retained chromatographically for 0.98 min, with a k' of 3.0. Calibrators were processed in pooled human urine obtained commercially. The peak signal intensity of the lowest calibrator was at least 2-fold higher than the matrix blank. Extracted ion chromatograms collected for blank urine and urine spiked with 0.0493 and 12.3 ng/mL HZ are provided in Figure 2. The pooled human urine used to prepare calibrators and QC materials should be screened prior to use to ensure that the signal intensity of the matrix blank does not exceed one-half of the signal intensity observed for the lowest calibrator of the method.

A small amount of HZ was detected in blank pooled urine, but was not observed above the lowest calibrator for the method. Table S1 includes the average peak areas (*n=4*) measured for blank urine, double blank urine (no internal calibrator added), water (deionized, 18 MΩ), and unfiltered water (deionized, 18 MΩ). The average peak area for blank urine was $(1.31 \pm 0.15) \times 10^4$ whereas the average peak area for double blank urine was $(1.24 \pm 0.14) \times 10^4$ confirming that the peak was not due to incomplete labeling of the internal standard. The average peak area for deionized water was $(4.10 \pm 0.78) \times 10^3$ as compared to unfiltered deionized water with an average peak area of $(4.89 \pm 1.03) \times 10^3$ confirming that the presence of hydrazine in the blank pooled urine is not from the filtration of the samples. The peak areas observed in deionized water are below the lowest reportable limit for this method. The small peak observed may be due to low amounts of hydrazine in the water, a possible

source since hydrazine is used in wastewater treatment and is a byproduct of chloramine disinfection [23–27].

Linearity, Precision and Accuracy

The peak area ratios of derivatized HZ to the derivative of isotopically-labeled HZ were linearly proportional to the ratio of the expected concentration of HZ in urine over the range of 0.0493–12.3 ng/mL. Over this linear range, the average (*n=21*) coefficient of determination, R^2 was 0.9985 ± 0.0013 with a line equation of $y = (0.147 \pm 0.010)\ x\ (-0.001 \pm 0.004)$. The lowest reportable limit for the method is the lowest calibrator, 0.0493 ng/mL. This corresponds to an on-column mass of 0.25 pg based on a 5.0 μL injection volume. The intraday and interday accuracy and precision values for HZ in Table 1 were determined by calculating the % error and % RSD for the standards run as unknowns in one day (n=4) and the standards run as calibrators during the 6 week characterization (n=21). For the 6-week method characterization, the accuracies across the range fell within 15% relative error. For the intraday accuracy, the lowest calibrator (0.0493 ng/mL) had a % error of 30% at 0.0643 ng/mL. This elevated error at the low end of the curve is representative of the characterized method in which calculated concentrations of S1 must fall between 0.0390 and 0.0736 ng/mL. The acceptable range of values for the calibrator was based on the full characterization, where a value was considered ‘passing’ if it was within 2 standard deviations of the average observed [33]. Three analysts participated in a 6 week QC characterization, analyzing no more than 2 calibration curves and corresponding QCs per day. The calibrators demonstrated ≤ 14% error with a corresponding %RSD ≤ 15%. All three levels of quality control materials were evaluated with ≤ 11% RSD for the 6 week validation. The method’s accuracy and precision follow the guidelines in the FDA’s guidance for bioanalytical method validation and thus show applicability for the analysis of clinical samples [34].

Recovery and Matrix Effects

To assess the recovery of HZ during sample filtration, calibrators were added to the pooled urine matrix either before filtration (“Processed”) or immediately prior to derivatization (“Unprocessed”). The “Processed” samples were representative of the sample preparation technique used for this analysis. The average peak areas (n = 4) from processed and unprocessed samples were used to calculate recovery. As shown in Table 2, recovery for both analytes was measured at low, mid, and high calibrator concentrations. The mean percentage recovery of HZ was 52 ± 9%, 45 ± 6%, and 52 ± 6%, at 0.123, 1.23 and 12.3 ng/mL, respectively. Although losses occur during the filtration recovery step, the loss is precise and normalized by isotope dilution across the linear range and therefore compliant with the FDA’s guidance for bioanalytical method validation [34]. Matrix effects were evaluated by making an injection of urine while infusing derivatized HZ [35]. No decrease was observed in the signal for the quantitation ion during the expected elution time of derivatized HZ, and no matrix effects were found to affect quantitation (Figure 3).

Stability and Ruggedness

The stability of HZ in urine was evaluated by allowing the HZ QC materials to stand for 4, 8 and 24 hours at −20, 4 and 37 °C prior to the addition of HZ*. The average response ratios

(n=3) were within 10% of the initial value for up to 24 hours at −20 °C and 8 hours at 4 °C. At 37 °C, the average response ratio dropped by greater than 15% at 4 hours. These data indicate that HZ stock solutions should be stored at −20 °C or less. Stability of HZ at the mid-level QC is provided in Figure 4, while low- and high-level QC stability is included in Figure S1. These data also indicate that HZ is not stable at human body temperature, and urine samples should be collected and frozen as soon as possible after suspected exposure. Storage effects were assessed by measuring the peak area ratios after three freeze-thaw cycles from −70 to 25 °C and were found to be stable within ± 10% of the theoretical value. Because the materials are stored in one-time use aliquots, stability up to three freeze-thaw cycles is adequate.

The ruggedness of the method was tested by assessing the changes in the following parameters: LC column temperature, collision energy, injection volume, LC flow rate, multiple column lots (*n=2*), and multiple MWCO filter lots (*n=3*). The ruggedness of the method was evaluated by comparing the calculated quality control concentration at the adjusted parameter to the calculated concentration obtained using the final method parameters. Each parameter was evaluated at a higher level and a lower level than the final method. For example, the flow rate, 500 μL/min, was also evaluated at 450 and 550 μL/min. The QC values for all ruggedness testing parameters fell within the acceptable 2σ range from the mean established during QC characterization. The collision energy was evaluated at 27.0 and 30.0 V as compared to the final method which uses a collision energy of 28.6 V, and method accuracy may be affected if the collision energy was changed significantly from the optimum value. The use of LC columns and filter plates from multiple product lots were not found to impact method accuracy.

Clinical Relevance

Low amounts of HZ, 0.23 ± 0.18 ng/mL, were detected in 99 of the 100 urine samples, and HZ was detected at 40.3 ng/mL in one of the samples. Low concentrations of HZ in the urine samples could be introduced from drinking water decontamination remnants [23–27] or in the case of the 40.3 ng/mL sample, a byproduct of pharmaceutical use [13–22]. The sample containing 40.3 ng/mL HZ in urine is more than an order of magnitude less than the lower end of the range previously reported for occupational exposure which was (2.7 ± 2.0) × 10^3 ng/mL. [29] Based on the same previous results, upper concentrations of occupational excretion could reach 9,000 ng/mL at three standard deviations of the average reported. Excretions exceeding 10,000 ng/mL are therefore expected in event-level exposure to hydrazine. Quantification of HZ concentrations in urine due to occupational exposure or an event-level exposure are anticipated to require a dilution of samples as described in this method. To quantify concentrations of HZ that are greater than the highest calibrator, a dilution of the sample urine with water was required. Dilution was determined to be appropriate for samples up to at least 1.23 × 10^5 ng/mL HZ. For 1.23 × 10^5 ng/mL, the %RE was ≤ 5% after dilution with water (Table 3). The corresponding %RSD for the 1.23 × 10^5 ng/mL sample was ≤ 5% (*n=8*, over 2 days). In the convenience set of individual samples, a 1:10 dilution was needed on one of the samples, which was determined to have a concentration of 40.3 ng/mL.

Conclusion

An analytical method was developed to address the need for a quantitative evaluation tool for human exposure to HZ in clinical samples. The described HPLC-MS/MS method provides quantitation of HZ in urine over a concentration range of 0.0493 to 12.3 ng/mL, which can be extended up to 1.23×10^5 ng/mL by diluting the sample prior to processing. This clinical range spans six orders of magnitude and can be used to monitor low-level to event-level exposures. The assay demonstrated accuracy within 15% error and precision within 15% RSD for calibration standards. Evaluation of QC materials demonstrated ≤ 11.2% RSD. With an analysis time of only 2 minutes per sample, this method provides a time-to-first result in less than 2.5 hours, including sample preparation, standard curve and QC sample analyses. The straightforward sample preparation and isocratic HPLC separation make this method easily employed by public health laboratories if needed. This work is the first quantitative HPLC-MS/MS assay reported for the measurement of HZ in human urine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Centers for Disease Control and Prevention and the Oak Ridge Institute for Science and Education. The authors would like to thank Ms. Chariety Sapp of the CDC's Incident Response Laboratory (IRL) for dispensing convenience set urine samples prior to analysis. The authors would also like to thank Dr. Patrick Dhooge of the New Mexico Department of Public Health for sharing insight on a previously developed GC-MS detection method.

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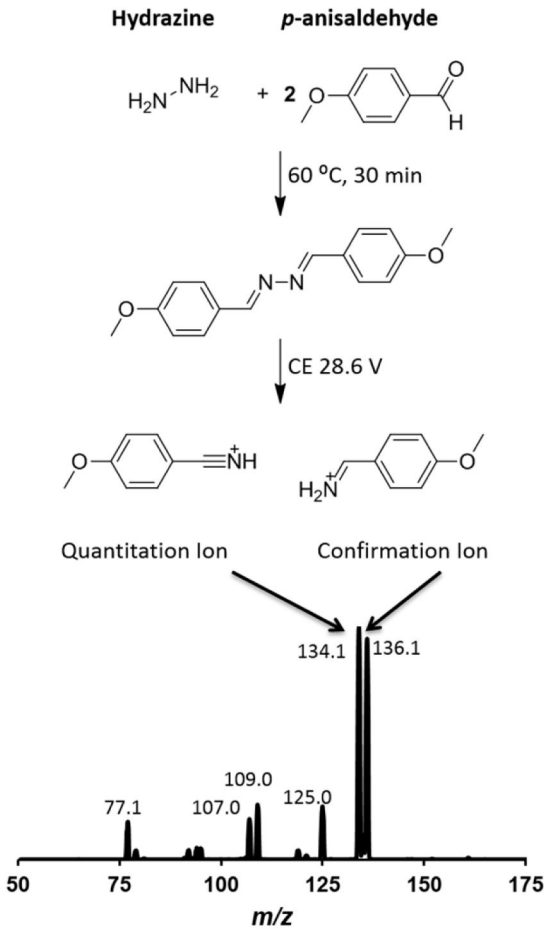

Figure 1.

(A) Derivatization of HZ with *p*-anisaldehyde was carried out under N_2 gas at 60°C. Derivatized HZ (*m/z* 269.1) was fragmented at a collision energy (CE) of 28.6 V, forming the two major product ions shown. (B) A product ion spectrum was obtained at CE 28.6 V, with the two largest fragments appearing at *m/z* 134.1 and 136.1. The most intense product ion, *m/z* 134.1, was used for quantitation of HZ, and m/z 136.1 was used as the confirmation ion.

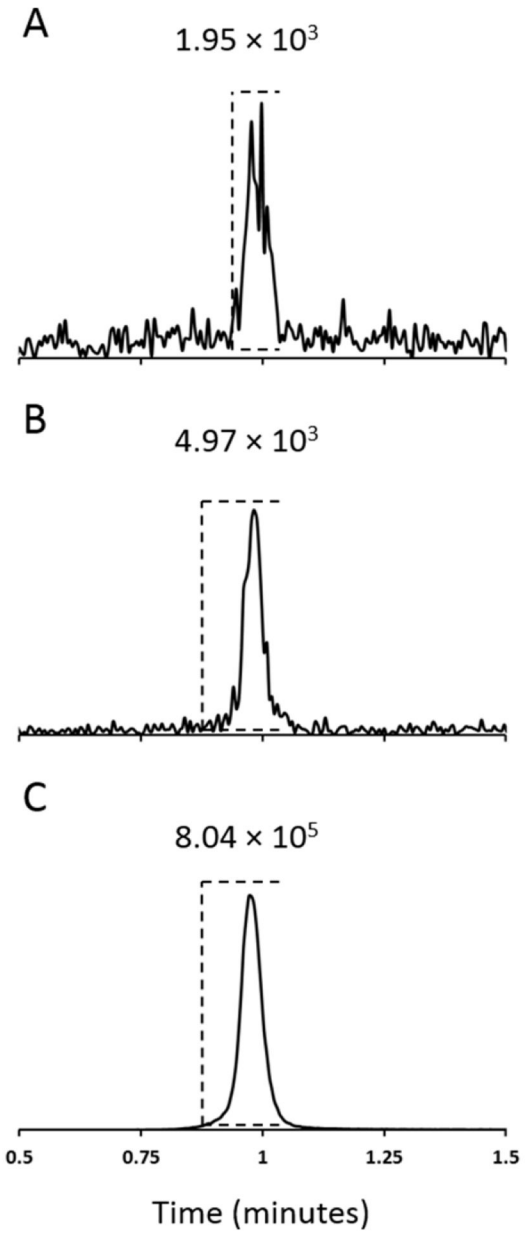

Figure 2.

Extracted ion chromatograms for transition *m/z* 269.1 → 134.1 of (A) unspiked pooled human urine, and human urine spiked with (B) 0.0493 ng/mL HZ and (C) 12.3 ng/mL HZ.

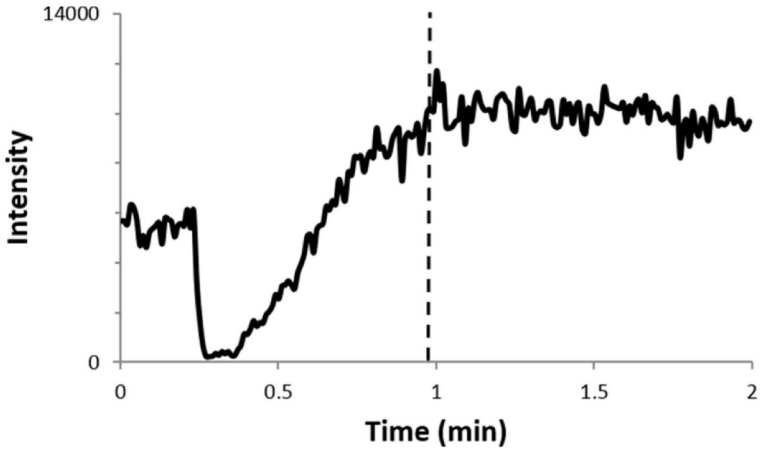

Figure 3.

Evaluation of matrix effects. A 5 μL injection of 1:1 pooled urine:acetonitrile was injected while infusing derivatized HZ. Matrix effects were not observed at the expected elution time of HZ in the extracted ion chromatogram for the transition *m/z* 269.1 → 134.1.

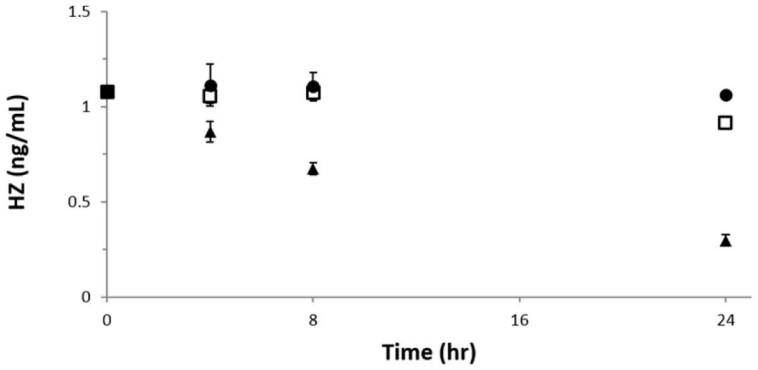

Figure 4.

Stability of HZ in human urine for mid-level quality control material (QM) monitored at (-●-) −20 °C, (-□-) 4 °C and (-▲-) 37 °C. Plots representative of QL and QH are included in the supplementary data. Error bars represent standard deviation (*n=3*). These data indicate that HZ stock solutions should be stored at −20 °C or less.

Table 1

Intraday (*n=4*) and interday (*n=21*) accuracy and precision for the determination of hydrazine in human urine.

	Intraday[a]			Interday[b]		
Standard concentration (ng/mL)	**average (ng/mL)**	**% error[c]**	**% RSD[d]**	**average (ng/mL)**	**% error[c]**	**% RSD[d]**
0.0493	0.0643	30	7.5	0.0563	14	15
0.123	0.140	14	6.6	0.117	−4.7	11
0.493	0.429	−13	2.1	0.462	−6.2	11
1.23	1.17	−4.7	3.8	1.16	−5.4	8.6
4.93	5.00	1.4	1.3	5.03	2.0	6.5
12.3	11.8	−4.5	2.9	12.3	0.039	3.0

[a] Intraday values were determined for the samples run as unknowns within one day (*n=4*)

[b] Interday values were caluculated from the complete method validation for the samples run as calibrators (*n=21*)

[c] [(experimental concentration-theoretical concentration)/theoretical concentration] × 100

[d] standard deviation/average × 100

Table 2

Recovery of HZ from human urine spiked with HZ before (processed) and after (unprocessed) filtration at low-, mid- and high-level analyte concentrations, n=4 for each.

	Processed (n=4)	Unprocessed (n=4)	
HZ conc. (ng/mL)	**mean area ± SD**	**mean area SD**	**% Recovery[a] SD[b]**
0.123	$(1.8 \pm 0.3) \times 10^4$	$(3.5 \pm 0.4) \times 10^4$	52 ± 9
1.23	$(2.0 \pm 0.3) \times 10^5$	$(4.4 \pm 0.1) \times 10^5$	45 ± 6
12.3	$(2.1 \pm 0.2) \times 10^6$	$(4.0 \pm 0.2) \times 10^6$	52 ± 6

[a] % Recovery = [(pk area for processed sample)/(pk area for unprocessed sample)] * 100

[b] $SD = \%recovery*[(SD_{un}/mean_{un})^2 + (SD_{proc}/mean_{proc})^2]^{1/2}$

Table 3

Accuracy and precision (n=8 for each concentration) for dilution of urine spiked with concentrations of HZ above the highest calibrator (> 12.3 ng/mL).

Initial conc (ng/mL)	Dilution factor	Calculated conc (ng/mL)	Measured conc (ng/mL)	% RSD	% Error
49.3	1.00×10^1	4.93	4.50	4.1	−8.7
123	2.50×10^1	4.93	4.66	2.1	−5.4
493	1.00×10^2	4.93	4.07	1.2	−17
1.23×10^3	2.50×10^2	4.93	4.07	2.9	−17
4.93×10^3	1.00×10^3	4.93	4.87	2.1	−1.2
1.23×10^4	2.50×10^3	4.93	5.04	2.0	2.4
4.93×10^4	1.00×10^4	4.93	5.07	2.6	2.9
1.23×10^5	2.50×10^4	4.93	5.17	4.5	4.9